CHARACTERIZATION OF MAJOR COMPONENTS IN BARKS FROM FIVE CANADIAN TREE SPECIES

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Abstract. In this study, the major components in barks from five Canadian tree species and their chemical and biological properties were characterized. The extractives soluble in hexane, ethanol, and 1% NaOH solution were measured through successive extractions. Total phenolic content was determined by the Folin-Ciocalteu method, antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl-free radical scavenging assay, and the characteristics of functional groups were analyzed by Fourier transform IR spectroscopy. The formaldehyde-condensable polyphenols were estimated with the Stiasny method. Lignin and holocellulose contents were determined by gravimetric method. Results showed that the amounts of extractives soluble in the three solvents varied significantly with bark species. Lodgepole pine bark contained the highest content of hexane-soluble extractives (15.0%), and aspen bark contained a very high content of ethanol solubles (22.3%). The 1% NaOH solubles ranged from 20.5 to 35.5% of the original bark. Except balsam fir, the total phenolic contents of ethanol solubles were between 200 and 300 mg equivalent catechin per gram of extract. The ethanol-soluble extractives from lodgepole pine bark and sugar maple bark had considerably high antioxidant potential; their IC_{50} values were about 11 μg/mL. The barks of softwood species contained a higher amount of formaldehyde-condensable polyphenols than those of hardwood species included in this study.

Keywords: Barks, extractives, Canadian tree species, chemical composition, total phenolics, formaldehyde-condensable polyphenols, antioxidant activity, FT-IR.

INTRODUCTION

Every year, a large amount of bark residues is produced by the Canadian forest and pulp and paper industries. These low-value byproducts have not been used efficiently for higher value applications. At present, a majority of bark is disposed of by burning or landfilling with only a small amount of bark used for industrial fuel and garden ground cover (Troughton 1995). Regarding the landfilling of bark residues, there have been serious concerns about groundwater pollution (Sweet and Fetrow 1975). Therefore, there is a significant benefit in exploring new ways to use these bark residues for value-added applications.

Bark is a complex biomass material of various chemical constituents including mainly polysaccharides and lignin and various extractives. Compared with wood, bark contains a much higher content of extractives that is composed of a diverse group of chemicals, eg fats, waxes, sterols, terpenes and terpenoids, phenolics, flavones and flavonoids, and polyphenols (tannins and polyphenolic acids) (Kurth 1947; Harkin and Rowe 1971; Fengel and Wegener 1984). These various types of chemical compounds from bark could be used in different industrial areas such as wood preservatives (Borgin and Corbett 1974),

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adhesives (Vazquez et al 1989; Miyazaki and Hirabayashi 2010), and medicines (Sofowora 1996) depending on their chemical and biological properties. Because bark is a renewable biomass resource, using chemicals from bark to replace petroleum-derived products can also bring about significant environmental benefits.

Recent efforts on using bark of some Canadian commercial tree species include using lodgepole pine bark for manufacturing biobased phenol–formaldehyde resins (Zhao et al 2010, 2013) and polyurethane foams (Zhao et al 2012; D'Souza and Yan 2013). In addition, Diouf et al (2009) measured the antioxidant properties and polyphenol contents of water extractives from aspen bark. Pakdel et al (2002) studied the extraction of betulin, a triterpenoid compound with antitumor activity, from white birch bark by a vacuum pyrolysis method. Yuan et al (2011) identified the chemical structures of four phenolic glycosides from sugar maple bark. Ross et al (1996) analyzed the chemical composition of the bark oil of balsam fir. However, a systematic analysis of chemical compositions of bark from common Canadian species and a comparison of the biological activities of the bark extractives from these species have not been reported. To identify suitable industrial chemical products that can be obtained from bark resources, it is essential to understand their composition and chemical and biological characteristics through a comprehensive analysis. In this study, major components in the barks of five selected Canadian tree species, including soluble extractives in various solvents, lignin, and holocellulose contents, were determined. Furthermore, analyses combining chemical characterization (eg total phenolics and formaldehyde-condensable polyphenols) with biological characterization (eg antioxidant activity) of the extractives from the barks were also conducted to further understand their use potentials.

MATERIALS AND METHODS

Bark Samples

Air-dried barks from five Canadian tree species, lodgepole pine (*Pinus contorta*), trembling aspen (*Populus tremuloides*), white birch (*Betula papyrifera*), sugar maple (*Acer saccharum*), and balsam fir (*Abies balsamea*), were collected from different sawmills in British Columbia or Quebec provinces of Canada. The bark samples were ground with a Wiley mill, and the fraction between 35 and 70 mesh was chosen for successive extractions with various solvents and chemical analysis.

Extractions

A portion of bark meal (10.00 g, ≈10% MC) for each bark species was Soxhlet-extracted successively with hexane and absolute ethanol for 10 h. The solvent in the extraction solution was removed with a rotary evaporator. The residue was further dried in a vacuum oven at room temperature overnight to give hexane solubles and ethanol solubles. The extract yields were calculated as weight percentages of original oven-dried bark meals used for extraction.

The extracted bark meal was finally extracted with 1% NaOH solution following procedures described in ASTM (2007): The ethanol-extracted bark was placed in a tall-form beaker to which a calculated volume of 1% NaOH solution (bark to NaOH solution ratio: 1:50 [w/v]) was added. After stirring well, the covered beaker was put in a steadily boiling water bath for 1 h and contents were stirred several times at an interval of 10 min. At the end of 1 h, the mixture was filtered by suction on a pretared fritted-glass crucible of medium porosity and the bark residue retained in the crucible was washed with hot water. The filtrate solution was collected for further determining formaldehyde-condensable polyphenol content. The extracted bark residue (regarded as extractive-free bark) was then washed with 10% acetic acid and then thoroughly washed with hot water, dried to constant weight at 105°C, cooled in a desiccator, and weighed. The yield of 1% NaOH solubles was calculated according to the weight difference ($W_{NaOH\text{-}solubles}$) of bark samples prior to and after treatment with the NaOH solution and expressed as a percentage of the weight of original bark.

Total Phenolic Compounds in Ethanol-Soluble Extracts

Determination of total phenolic compounds in the ethanol-soluble extracts was carried out based on the Folin–Ciocalteu method with slight modifications (Huang et al 2009). Five milligrams of extract powder was dissolved in 50 mL of ethanol. A portion of this ethanol solution (1000 μL) was transferred to a 10-mL flask and evaporated to remove ethanol. The residue in the flask was dissolved with 3 mL of water, and the solution was quantitatively transferred to a 10-mL volumetric flask. One milliliter of Folin–Ciocalteu phenol reagent was added, and the flask was shaken vigorously. Then 5 mL of 20% sodium carbonate aqueous solution was added, and the mixture was made up to 10 mL with water and shaken thoroughly again. After 20 min of incubation, the absorbance of the mixture was measured at 735 nm. The working curve was determined using (+)-catechin (analytical standard; Sigma-Aldrich, St. Louis, MO) as a standard. The content of total phenolic compounds was expressed as the weight of total catechin equivalents in the ethanol-soluble extract (mg/g).

Antioxidant Assay for Ethanol-Soluble Extracts

The antioxidant assay for scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was conducted according to the method described by Huang et al (2009). The reaction mixtures were prepared by mixing 1000 μL of 0.1 mM DPPH solution in ethanol, 450 μL of 0.05 M Tris-HCl buffer (pH 7.6), and 50 μL of test sample ethanol solution (final concentrations were 1, 2, 3, 5, 10, 20, 30, 40, 50, and 100 μg/mL, respectively) or ethanol (used as control). Decrease of the DPPH-free radical was measured by recording the absorbance at 517 nm exactly 30 min after each extract solution was added. Butylated hydroxytoluene (BHT, analytical standard; Sigma-Aldrich) was used as a positive reference in the test. The inhibition ratio was expressed as a percentage after being calculated from Eq 1:

$$\%\text{Inhibition} = \left[(A_c - A_s)/A_c\right] \times 100\% \quad (1)$$

where A_c is absorbance of the control solution, and A_s is absorbance of the test sample solution. The inhibitory concentration that caused 50% scavenging of the DPPH radical (IC_{50}) was estimated based on the plot of inhibition vs final concentration of the test samples.

Fourier Transform IR Spectroscopy Analysis for Ethanol-Soluble Extracts

Fourier transform IR spectroscopy (FT-IR) spectra of the ethanol-soluble extracts were measured using the KBr pellet technique (1.0 mg of extract sample dispersed in 150 mg of KBr) on a TENSOR 27 FT-IR spectrometer (Bruker Optics, Billerica, MA).

Formaldehyde-Condensable Polyphenols in 1% NaOH Extract and Stiasny Number

The amount of formaldehyde-condensable polyphenols in 1% NaOH extract and its Stiasny number were determined using a modified procedure according to Garro Galvez et al (1997). The filtrate solution from 1% NaOH extraction was cooled to room temperature and weighed (W_0). After 10.00 (W_1) g of this solution was transferred to a 50-mL flask, 1 mL of 10 M HCl and 2 mL of formaldehyde (37%) were added and the mixture was heated under reflux for 30 min. The reaction mixture was filtered while hot through a pretared fritted-glass crucible of medium porosity. The precipitate was washed with hot water, dried at 105°C overnight, and weighed (W_2). The total yield of formaldehyde-condensable polyphenols (W_{FC}) was calculated according to Eq 2 and expressed as a percentage of the weight of original bark:

$$W_{FC} = [W_0/W_1] \times W_2 \quad (2)$$

Stiasny number, defined as the weight in grams of formaldehyde-condensable polyphenols

in 100 g of 1% NaOH extract, can be calculated according to Eq 3.

$$\text{Stiasny number} = [W_{FC}/W_{NaOH-solubles}] \times 100 \quad (3)$$

Chemical Analysis of Extractive-Free Bark

The content of acid-insoluble lignin (Klason lignin) in extractive-free bark (the residue of bark after 1% NaOH extraction) was determined according to a modified procedure (Effland 1977; Puls 1993). A portion of the oven-dried sample (200 mg) was weighed into a hydrolysis tube (short test tube) for acid hydrolysis. All samples were run in triplicate. Each sample was hydrolyzed with 2 mL of 72% sulfuric acid for 1 h at 30°C by incubating the test tube in a 30 ± 0.5°C water bath with constant stirring using a glass rod. The reaction was stopped by the addition of 6 mL of distilled water. The sample was then quantitatively transferred into a 125-mL Erlenmeyer flask using 50 mL of distilled water. The flask was then closed with a glass cover and autoclaved at 120°C for 40 min. The solid residue (Klason lignin) was filtered using a pretared fritted-glass crucible and washed extensively with distilled water. Then the crucible and its contents were dried at 105°C overnight, cooled in a desiccator, and weighed. The holocellulose content was estimated by deducting Klason lignin from the extractive-free bark.

RESULTS AND DISCUSSION

Chemical Composition

The barks from five Canadian tree species were analyzed to determine their chemical composition, including the content of hexane, ethanol, and 1% NaOH extractives in a sequential extraction, and the contents of lignin and holocellulose. The results indicate that the barks contained a higher content of extractives than wood, and the amounts of extractives from the five tree species vary significantly from species to species (Table 1). The total amounts of extractives obtained by the successive extractions with hexane, ethanol, and 1% NaOH ranged from 28 to 62%.

Hexane extractives. The extractives soluble in hexane include fatty acids, fats (esters of fatty acids with glycerol), oils (liquid fats), waxes (esters of fatty acids with saturated straight-chain alcohols), resins and resin acids (terpenes and their derivatives), and sterols (Harkin and Rowe 1971; Hillis 1987; Ngueho Yemele et al 2008). Among the five bark samples, lodgepole pine bark, aspen bark, and balsam fir bark contained quite high amounts of hydrophobic substances. The hexane-soluble extractives contents of these three barks were 15.0, 8.6, and 8.9%, respectively. The hexane-soluble extractives contents in white birch bark and sugar maple bark were very low (about 2%).

Ethanol extractives. Ethanol extraction may remove coloring matter, simple phenols, phenolic acids, and their esterified products with glucose, polyols and other phenols, flavones and their derivatives, stibenes and their derivatives, lignans, quinones, simple polyphenols and their glycosides, tannins, and mono- and disaccharides (Harkin and Rowe 1971; Fengel and Wegener 1984; Hillis 1987). According to Table 1, the amounts of ethanol-soluble extractives from the five tree species also differed significantly, ranging from 3.5 to 22.3%. Aspen bark contained a

Table 1. Chemical composition of barks (percentage based on original oven-dried bark before extracted).

Bark	Hexane solubles (%)	Ethanol solubles (%)	1% NaOH solubles (%)	Holocellulose (%)	Lignin (%)
Lodgepole pine	15.0	11.7	35.5	28.9	8.9
Aspen	8.6	22.3	26.2	30.2	12.7
White birch	1.9	3.1	23.7	44.0	27.3
Sugar maple	2.3	5.5	20.5	44.8	26.9
Balsam fir	8.9	4.5	32.6	37.9	16.1

very high content of ethanol-soluble extractives (more than 22%). Lodgepole pine bark also contained a high amount of ethanol-soluble extractives (11.7%). Amounts of ethanol-soluble extractives in the other three barks were less than 6%.

One percent NaOH extractives. One percent NaOH extraction removed the extractives from bark included condensed tannins (polymerized polyphenols), some bark lignin, low-molecular-weight carbohydrates (mainly hemicelluloses), suberin fragments, proteins, alkaloids, and ash (Harkin and Rowe 1971; Fengel and Wegener 1984; Hillis 1987; Kofujita et al 1999). After having been successively extracted with hexane and ethanol, the contents of 1% NaOH-soluble extractives from the five barks were still very high, ranging from 20.5 to 35.5%. In addition to suberin materials that were not contained in wood, the high amounts of 1% NaOH-soluble extractives suggested that bark contained more condensed tannins than wood. The results also indicated that the amounts of 1% NaOH extractives from softwood bark, lodgepole pine (35.5%) and balsam fir (32.6%), were more than those obtained from the three hardwood barks, which ranged from 20.5 to 26.2%.

Lignin and holocellulose contents. In this study, the content of Klason lignin was determined in 1% NaOH extracted barks. In this way, overestimates of lignin amounts in barks could be avoided (Kiefer and Kurth 1953). As indicated in Table 1, based on the original oven-dried bark, there is quite a big variation in the Klason lignin content among bark from the five tree species. Lodgepole pine bark contained the lowest Klason lignin content (only 8.9%). Klason lignin contents in the bark of aspen (12.7%) and balsam fir (16.1%) were also quite low. Bark from white birch and sugar maple contained the highest amount of lignin, which accounted for about 27% of the original bark mass. Because of a high total amount of extractives in all five barks, holocellulose contents ranged from 28.9 to 44.8%, which were obviously lower than that in wood.

Total Phenolics and Antioxidant Activity of Ethanol Extractives

Total phenolic contents of ethanol-soluble extractives and their IC_{50} values of antioxidant assay for scavenging DPPH radical are shown in Table 2. The results indicate the total phenolic contents of the ethanol extractives from all the bark species except balsam fir ranged between 200 and 300 mg/g extract. The ethanol-soluble extract from balsam fir bark contained the least phenolic compounds, and its total phenolic content was only 131 mg/g extract.

The results of antioxidant activity showed all ethanol-soluble extracts could scavenge DPPH radicals to some extent. In particular, the ethanol-soluble extracts from barks of lodgepole pine and sugar maple showed considerable antioxidant potential. Their IC_{50} values were 11.0 and 11.3 μg/mL, respectively. The IC_{50} value of BHT, a synthetic antioxidant primarily used as an antioxidant additive for food and cosmetics and a positive control for DPPH assay in this study, was 5.0 μg/mL. Thus, the antioxidant activities of the ethanol extracts from lodgepole pine and sugar maple barks were equivalent to about 50% of BHT.

Fourier Transform IR Spectroscopy Analysis of Ethanol Extractives

FT-IR spectra of the ethanol extractives are shown in Fig 1. As can be seen from the spectra, all the ethanol extractives have a strong hydrogen-bonded

Table 2. Total phenolics and antioxidant activity of ethanol-soluble extractives.

Bark	Total phenolics[a] (mg/g extract)	IC_{50} of antioxidant activity[b] (μg/mL)
Lodgepole pine	279	11.0
Aspen	253	33.6
White birch	297	38.7
Sugar maple	236	11.3
Balsam fir	131	15.3

[a] Expressed as the weight of total catechin equivalents in the ethanol solubles.
[b] Oxidant concentration required for 50% scavenging of the initial DPPH-free radical.
Butylated hydroxytoluene (BHT): positive control for antioxidant assay, IC_{50} = 5.0 μg/mL.
DPPH, 1,1-diphenyl-2-picrylhydrazyl.

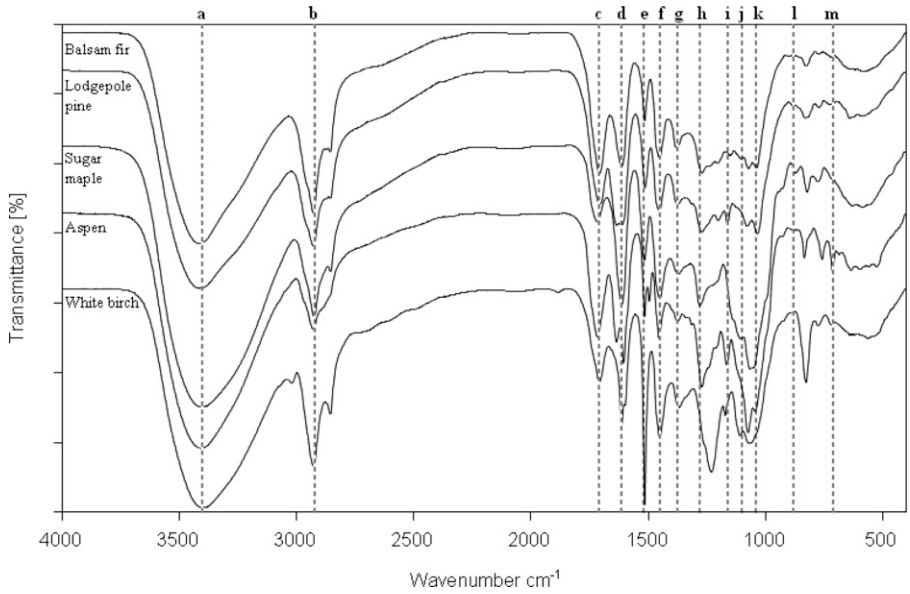

Figure 1. Fourier transform IR spectra of ethanol-soluble extractives from the barks of five Canadian tree species.

O-H stretching absorption in the region of 3400-3100 cm^{-1} (a) with a prominent C-H stretching absorption band at 2920 cm^{-1} (b) and a unconjugated C=O stretching adsorption of carboxyl and acetyl groups apparent at 1710 cm^{-1} (c). Some of the well-defined peaks in the fingerprint region 1700-600 cm^{-1} caused by various functional groups could have been assigned according to Yazaki and Hillis (1977), Silverstein et al (1981), and Ku and Mun (2007): 1612 cm^{-1} (d) for symmetric stretching of aromatic ring and C=C alkene groups; 1515 cm^{-1} (e) for skeletal vibrations of phenyl rings; 1452 cm^{-1} (f) for the asymmetric stretching of aromatic rings and for CH_2 deformation of alkane groups; 1374 cm^{-1} (g) for C-H bending modes; 1272 cm^{-1} (h) for aromatic O-H; 1162 cm^{-1} (i) for C-O-C vibration; the peaks at1110-1040 cm^{-1} region (j-k) for C-O stretching of alcohols; and the peaks at 880-720 cm^{-1} region (l-m) for =C-H bending of alkene groups. The bands falling in the fingerprint region 1700-600 $cm^{-1}$are complex and might contain contributions from various types of extractive compounds.

Based on the designation for these peaks in the fingerprint region, in general, all ethanol extractives contain aromatic rings, aromatic O-H groups, and alcohol groups. The relative intensities of adsorption peaks for these functional groups are different for each extractive sample. Ethanol extractives from white birch bark have the strongest phenyl ring adsorption at 1515 cm^{-1} (e) indicating that it contains the highest phenolic compounds, which is in accordance with results of the total phenolic determination. The absorption peak at 1272 cm^{-1} (h) reveals that the content of aromatic O-H groups in the ethanol extractives from aspen, sugar maple, and lodgepole pine barks is higher than that of balsam fir and white birch barks. Conversely, the absorption peaks in the region of 1110-1040 cm^{-1} (j-k) suggest that the ethanol extractives from sugar maple, aspen, and white birch barks contain more -C-OH alcohol groups than those from balsam fir and lodgepole pine barks.

Formaldehyde-Condensable Polyphenols in 1% NaOH-Soluble Extractives

Bark contains more polyphenols than wood. Some of these polyphenols cannot be extracted with neutral organic solvents or hot water but

Table 3. Formaldehyde-condensable polyphenols in 1% NaOH-soluble extractives.

Bark	Formaldehyde-condensable polyphenols[a] (%)	Stiasny number[b]
Lodgepole pine	8.3	23.9
Aspen	3.7	14.0
White birch	6.8	28.9
Sugar maple	2.6	12.5
Balsam fir	12.5	38.4

[a] Based on the original oven-dried bark before extraction.
[b] Defined as the weight in grams of formaldehyde-condensable polyphenols in 100 g of 1% NaOH-soluble extractives.

are soluble in 1% NaOH at an elevated temperature (Browning 1967; Fengel and Wegener 1984; Ona et al 1995). The bark extractives containing formaldehyde-condensable polyphenols could be used for adhesive formulations or for substituting phenol partly in making phenol–formaldehyde resins depending on the percentage content (Stiasny number) of formaldehyde-condensable polyphenols in extractives (Vazquez et al 1989, 2001). The results indicated that the amounts of formaldehyde-condensable polyphenols in the 1% NaOH extracts ranged from 2.6 to 12.5% based on the original oven-dried bark (Table 3). The 1% NaOH-soluble extracts from softwood barks (lodgepole pine and balsam fir) contained a higher content of formaldehyde-condensable polyphenols than those of the hardwood barks (aspen, white birch, and sugar maple). The Stiasny numbers of the 1% NaOH extracts from lodgepole pine, white birch, and balsam fir barks were relatively higher than those of the extracts from aspen and sugar maple barks. These results suggested that the 1% NaOH extracts from these three barks might be more suitable to be used for making adhesives.

CONCLUSIONS

Among the five Canadian commercial tree species barks analyzed in this study, lodgepole pine bark contained the highest content of hexane-soluble hydrophobic substances (15%), whereas aspen bark contained the highest content of ethanol-soluble extractives (22%). After successively extracted with hexane and ethanol, the barks from the two softwood species (lodgepole pine and balsam fir) contained a higher amount of 1% NaOH-soluble substances than the other three hardwood species (aspen, white birch, and sugar maple). The ethanol extracts from lodgepole pine and sugar maple barks exhibited a considerable antioxidant capacity. The 1% NaOH extracts from lodgepole pine, white birch, and balsam fir barks contained a relatively higher content of formaldehyde-condensable polyphenols compared with other species. These results provide clues toward identifying optimum strategies for using these bark resources for high value-added applications.

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